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JC03 Rec'd PCT/PTO 11 JAN 2002

BAKER BOTTS L.L.P

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35.U.S.C. 371

EXPRESS MAIL LABEL No.	DATE
EF 377 398 644 US	January 11, 2002

ATTORNEY'S DOCKET NO. 34920-PCT-USA 072667.0179

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR 1.5) 10/030829

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/02052	July 13, 2000	July 16, 1999

TITLE OF INVENTION: NOVEL SGS3 PLANT GENE AND USES THEREOF

APPLICANT(S) FOR DO/EO/US: Christophe BECLIN, Taline ELMAYAN, and Hervé VAUCHERET

Applicant herewith submits to the United States Designated /Elected Office (DO/EO/US) the following items and other information:

1. ☑ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☑ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I).
4. ☑ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☑ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☑ has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☑ A copy of the International Search Report (PCT/ISA/210)
   a. ☑ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☑ A copy of the International Preliminary Examination Report (PCT/IPEA/409)
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [ ] Other items or information:
    a. [ ] a copy of the International Search Report (PCT/ISA/210)
    b. [ ] a copy of the International Preliminary Examination Report (PCT/IPEA/409)

10/030829
531 Rec'd PCT/PTO 11 JAN 2002

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/02052	July 13, 2000	July 16, 1999

17. [ ] The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5):

Neither international preliminary examination fee (37 CFR 1.482)

Nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO (1.492(a)(3)) $1,040

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO (1.492(a)(5) $890.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO(1.492(a)(2)) $740.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) (1.492(a)(1)) $710.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $ 100.00

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT				= $	890
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [✓] 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).				$	130
Claims	**Number Filed**	**Number Extra**	**Rate**	$	
Total Claims	61 -20=	41	X $ 18.00	$	738
Independent Claims	4 -3=	1	X $ 84.00	$	84
Multiple dependent claim(s) (if applicable)			+ $280.00	$	280
TOTAL OF ABOVE CALCULATIONS				= $	2,122
Reduction by ½ for filing by small entity, if applicable.				$	
SUBTOTAL				= $	2,122
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [✓] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	130
TOTAL NATIONAL FEE				= $	2,252
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED				= $	2,252
				Amt. refunded	$
				charged	$

a. [ ] A check in the amount of $ 2,252.00 to cover the above fees is enclosed.

b. [ ] Please charge our Deposit Account No. 02-4377 in amount of $_____ to cover the above fees. A copy of this sheet is enclosed.

c. [✓] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4377. A copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
Alicia A. Russo
BAKER BOTTS L.L.P.
30 Rockefeller Plaza
New York, New York 10112-4498

Alicia A. Russo

Attorney: Alicia A. Russo PTO Reg: 46,192

January 11, 2002
Date

A34920-PCT-USA 072667.0179
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Christophe Beclin et al.			
Serial No.	:	10/030,829	Examiner	:	TBA
Filed	:	January 11, 2002	Group Art Unit	:	TBA
For	:	NOVEL SGS3 PLANT GENE AND USES THEREOF			

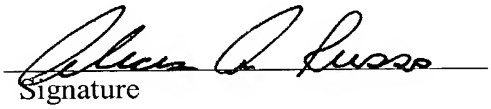
PRELIMINARY AMENDMENT

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

April 11, 2002
Date of Deposit

Alicia A. Russo
Name

46,192
PTO Reg. No.


Signature

April 11, 2002
Date of Signature

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants respectfully request entry of the following amendments prior to examination on the merits pursuant to 37 C.F.R. §1.115(b)(2)(iii). Applicants enclose herewith an English translation of the international application pursuant to 37 C.F.R. §1.495(c)(1). Applicants also enclose a Substitute Sequence Listing in paper and electronic form, a Combined Declaration and Power of Attorney, and an Assignment and enclose the fees required pursuant to

37 C.F.R. §1.121(h). The fees required pursuant to 37 C.F.R. §1.16(e) and 37 C.F.R. §1.492(f) have been submitted previously.

IN THE SPECIFICATION

Please **delete** the Sequence Listing presently of record and substitute, therefor, the attached Substitute Sequence Listing.

Please **amend** the paragraph beginning on page 4, line 14 (of the English translation) and ending on page 4, line 18 with the following rewritten paragraph:

Description of the Sequence Listing

SEQ ID NO:1: *SGS3* gene of *Arabidopsis thaliana*.

SEQ ID NO:2: cDNA of the *SGS3* gene of *Arabidopsis thaliana*.

SEQ ID NO:3: *SGS3* polypeptide of *Arabidopsis thaliana*.

SEQ ID NO:4: Primer p356AD'.

SEQ ID NO:5: Primer p356Y'.

Please **amend** the paragraph beginning on page 47, line 1 (of the English translation) and ending on page 47, line 22 with the following rewritten paragraph:

The DNA sequence which was inserted at the BamHI site of the pBin+ plasmid and which had led to the isolation of the bacterial strain 356 was determined. Subclones of the 356 clone were produced in the pBin+ vector and the same *sgs3-2* 2a3 line was transformed with these subclones in order to determine those

capable of restoring the function of the *SGS3* gene. The smallest subclone capable of restoring this function constitutes the *SGS3* gene such as it is described in this disclosure. It was possible to predict the ORF of *SGS3* by computer analysis. The sequence of the cDNA containing the ORF of the *SGS3* gene, and therefore the position of the promoter, terminator and intronic sequences of *SGS3*, were verified after having isolated and cloned this sequence. In order to isolate, we first performed a reverse transcription reaction using *Arabidopsis thaliana* total RNA. We then performed a PCR reaction on this pool of cDNA using the pair of primers p356AD' (AAAATGAGTTCTAGGGCTGGTCC; SEQ ID NO:4) and p356Y' (GTCTCAATCATCTTCATTGTGAAGGCC; SEQ ID NO:5). These primers are located at the 2 ends of the ORF of SGS3. This PCR product was cloned and sequenced.

IN THE CLAIMS

Please **cancel** claims 1-22.

Please **add** the following new claims:

23. (NEW) An isolated nucleic acid comprising a nucleotide sequence having at least 80% homology to a reference nucleotide sequence wherein the reference sequence is selected

from the group consisting of nucleotides 1-695 of SEQ ID NO:1, SEQ ID NO:1, SEQ ID NO:2, and the complements thereof.

24. (NEW) The isolated nucleic acid of claim **23** wherein said nucleotide sequence is at least 90% homologous to the reference sequence.

25. (NEW) The isolated nucleic acid of claim **24** wherein said nucleotide sequence is at least 95% homologous to the reference sequence.

26. (NEW) The isolated nucleic acid of claim **25** wherein said nucleotide sequence is at least 98% homologous to the reference sequence.

27. (NEW) The isolated nucleic acid of claim **26** wherein said nucleotide sequence is at least 99% homologous to the reference sequence.

28. (NEW) The isolated nucleic acid of claim **23** wherein said reference sequence is nucleotides 1-695 of SEQ ID NO:1.

29. (NEW) The isolated nucleic acid of claim **23** wherein said reference sequence is selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.

30. (NEW) The isolated nucleic acid of claim **28** wherein said nucleic acid has promoter activity in a plant cell or a plant.

31. (NEW) An isolated nucleic acid comprising a nucleotide sequence having nucleotides 1-695 of SEQ ID NO:1.

32. (NEW) An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.

33. (NEW) The isolated nucleic acid of claim **32** wherein said nucleotide sequence is SEQ ID NO:1.

34. (NEW) The isolated nucleic acid of claim **32** wherein said nucleotide sequence is SEQ ID NO:2.

35. (NEW) The isolated nucleic acid of claim **29** wherein said nucleic acid restores an sgs3 mutant of *Arabidopsis thaliana*.

36. (NEW) An isolated polypeptide comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3.

37. (NEW) The isolated polypeptide of claim **36** wherein said amino acid sequence is at least 90% homologous to SEQ ID NO:3.

38. (NEW) The isolated polypeptide of claim **37** wherein said amino acid sequence is at least 95% homologous to SEQ ID NO:3.

39. (NEW) The isolated polypeptide of claim **38** wherein said amino acid sequence is at least 98% homologous to SEQ ID NO:3.

40. (NEW) The isolated polypeptide of claim **39** wherein said amino acid sequence is at least 99% homologous to SEQ ID NO:3.

41. (NEW) The isolated polypeptide of claim **36** wherein said polypeptide restores an sgs3 mutant or *Arabidopsis thaliana*.

42. (NEW) An isolated polypeptide comprising an amino acid sequence of SEQ ID NO:3.

43. (NEW) An isolated polypeptide comprising a fragment of a polypeptide having an amino acid sequence of SEQ ID NO:3 wherein said fragement has biological activity in a plant or plant cell.

44. (NEW) An expression cassette comprising:

a plant promoter;

a nucleic acid comprising a nucleotide sequence that is at least 80% homologous to SEQ ID NO:2; and

a plant terminator,

wherein said plant promoter is operably linked to said nucleic acid, and wherein said terminator is operably linked to said nucleic acid.

45. (NEW) An expression cassette comprising:

a plant promoter;

a nucleic acid comprising a nucleotide sequence that is at least 80% homologous to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2; and

a plant terminator,

wherein said plant promoter is operably linked to said nucleic acid, and wherein said terminator is operably linked to said nucleic acid.

46. (NEW) An expression cassette comprising:

a plant promoter having a nucleotide sequence that is at least 80% homologous to nucleotides 1-695 of SEQ ID NO:1,

a nucleic acid encoding a heterologous polypeptide, and

a plant terminator,

wherein said plant promoter is operably linked to said nucleic acid, and wherein said terminator is operably linked to said nucleic acid.

47. (NEW) An expression vector or transformation vector comprising a nucleic acid of claim **23**, **28**, or **29** or an expression cassette of claim **44**, **45**, or **46**.

48. (NEW) A process for transforming a host organism comprising contacting the host organism with either a nucleic acid of claim **23**, **28**, or **29** or an expression cassette of claim **44**, **45**, or **46**.

49. (NEW) A process for expressing a heterologous gene in a host organism comprising

contacting a host organism, comprising a heterologous gene, with an expression cassette comprising:

a plant promoter;

a nucleic acid comprising a nucleotide sequence that is at least 80% homologous to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2; and

a plant terminator.

50. (NEW) A process for expressing a heterologous gene in a host organism comprising contacting a host organism which comprises a heterologous gene, with a polypeptide comprising an amino acid sequence that is at least 80% homologous to SEQ ID NO:3.

51. (NEW) A transformed host organism comprising at least one nucleic acid of claim **23**, **28**, or **29** or an expression cassette of claim **44**, **45**, or **46**.

52. (NEW) An isolated nucleic acid that selectively hybridizes to a nucleic acid having a nucleotide sequence selected from the group consisting of nucleotides 1-695 of SEQ ID NO:1, SEQ ID NO:1, SEQ ID NO:2, and the complements thereof.

REMARKS

Applicants respectfully request entry of the following amendments prior to examination on the merits pursuant to 37 C.F.R. §1.115(b)(2)(iii). Applicants enclose herewith an English translation of the international application pursuant to 37 C.F.R. §1.495(c)(1). Applicants also enclose a Substitute Sequence Listing in paper and electronic form, a Combined Declaration and Power of Attorney, and an Assignment together with the fees required pursuant to 37 C.F.R. §1.21(h). The fees required pursuant to 37 C.F.R. §1.16(e) and 37 C.F.R. §1.492(f) have been submitted previously.

Claims 1-22 are pending. Claims 1-22 have been cancelled and new claims 23-52 have been added. Applicants assert that the new claims are fully supported by the application as orginally filed and, therefore, do not constitute new matter. Specifically, claims 23-52 are supported by, *inter alia*, original claims 1-22.

Rewritten paragraphs appear in the preceding "IN THE SPECIFICATION" section. Attached hereto is a marked-up version of the changes made to the specification paragraphs by the instant amendment captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" and is included pursuant to 37 C.F.R. §1.121(c)(ii). Should any discrepancies be discovered, the version presented in the preceding "IN THE SPECIFICATION" section shall take precedence.

A sequence listing in computer readable form has not previously been filed in this application. Nevertheless, both the electronic and paper sequence listing attached hereto are identified as "Substitute Sequence Listing."

I hereby state that the content of the paper and computer readable copies of the Substitute Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e), are the same. I hereby state that the content of the paper and computer readable copies of the Substitute Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(g), herein does not include new matter.

The Commissioner is hereby authorized to charge any fees due with this submission not otherwise enclosed herewith to Deposit Account No. 02-4377. Please credit any overpayment of fees associated with this filing to the above-identified deposit account. A duplicate of this page is enclosed.

Respectfully submitted,

April 11, 2002

Louis S. Sorell
PTO Reg. No. 32,439

Alicia A. Russo
PTO Reg. No. 46,192
Attorneys for Applicant

BAKER BOTTS, L.L.P.
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Enclosures

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

This marked-up version was prepared with DeltaView software (v2.5.163). In this section, added text is marked with double underlining. *e.g.* added text, and deleted text is marked by a single strikethrough, *e.g.* ~~deleted text~~.

IN THE SPECIFICATION

The paragraph beginning on page 4, line 14 (of the English translation) and ending on page 4, line 18 has been **amended** as follows:

Description of the Sequence Listing

SEQ ID ~~No.~~ NO:1: *SGS3* gene of *Arabidopsis thaliana*.

SEQ ID ~~No.~~ NO:2: cDNA of the *SGS3* gene of *Arabidopsis thaliana*.

SEQ ID ~~No.~~ NO:3: *SGS3* polypeptide of *Arabidopsis thaliana*.

SEQ ID NO:4: Primer p356AD'.

SEQ ID NO:5: Primer p356Y'.

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The DNA sequence which was inserted at the BamHI site of the pBin+ plasmid and which had led to the isolation of the bacterial strain 356 was determined. Subclones of the 356 clone were produced in the pBin+ vector and the same *sgs3-2* 2a3 line was transformed with these subclones in order to determine those

capable of restoring the function of the *SGS3* gene. The smallest subclone capable of restoring this function constitutes the *SGS3* gene such as it is described in this ~~patent~~disclosure. It was possible to predict the ORF of *SGS3* by computer analysis. The sequence of the cDNA containing the ORF of the *SGS3* gene, and therefore the position of the promoter, terminator and intronic sequences of *SGS3*, were verified after having isolated and cloned this sequence. In order to isolate, we first performed a reverse transcription reaction using *Arabidopsis thaliana* total RNA. We then performed a PCR reaction on this pool of cDNA using the pair of primers p356AD' (AAAATGAGTTCTAGGGCTGGTCC; SEQ ID NO:4) and p356Y' (GTCTCAATCATCTTCATTGTGAAGGCC; SEQ ID NO:5). These primers are located at the 2 ends of the ORF of SGS3. This PCR product was cloned and sequenced.

Novel *SGS3* plant gene and use thereof

The present invention relates to a novel *SGS3* plant gene and use thereof for preparing genetically modified plants.

Methods are known, from the state of the art, which make it possible to integrate heterologous genes into the genome of plants of various species. For the processes for transforming plant cells and for regenerating plants, mention will in particular be made of the following patents and patent applications: US 4,459,355, US 4,536,475, US 5,464,763, US 5,177,010, US 5,187,073, EP 267 159, EP 604 662, EP 672 752, US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318, US 5,204,253, US 5,405,765, EP 442 174, EP 486 233, EP 486 234, EP 539 563, EP 674 725, WO 91/02071 and WO 95/06128.

The level of expression of the heterologous gene will depend on various factors, including the locus of integration of the heterologous gene into the genome of the transformed plant and "silencing" phenomena. It is, in fact, known from the state of the art that the expression of a heterologous gene in a plant may be totally or partially inhibited in the descendents of the regenerated transformed plants, even

though said gene is expressed correctly in the regenerated plant directly derived from the transformed cell. The heterologous genes introduced may sometimes undergo epigenetic inactivation (inactivation accompanied by no sequence modification). When the genes exhibit homology with genes of the host organism, the inactivation may also affect the expression of these host genes and engender effects which are deleterious for the organism (co-inactivation). Two distinct inactivation mechanisms have been demonstrated in higher plants, resulting either in blocking of transcription (transcriptional inactivation) or in RNA degradation (post-transcriptional inactivation).

These inactivation phenomena, accidentally revealed by transgenesis, certainly reflect fundamental processes for the epigenetic control of gene expression, and their study therefore constitutes an original means of access to understanding the regulatory mechanisms used during plant development. The demonstration of these phenomena raises, moreover, many questions regarding the use of transgenic plants both for variety improvement programs and for molecular physiology studies.

Thus, monolocus homozygous plants obtained with a gene encoding the GUS protein under the control of the promoter CamV 35S (35S-UidA) have exhibited inactivation of the transgene, regardless of the number of copies of the transgene inserted at the locus. The

phenomenon occurs during the development of each generation, indicating meiotic reversibility. Haploid plants derived from culturing anthers of inactivated homozygous transformants carrying a single copy of the transgene have shown reactivation of the gene followed by inactivation during development, suggesting that meiosis is necessary for triggering the reactivation process, but that the triggering of the inactivation during development does not require fertilization, and does not result from interaction between various copies of the transgene. Finally, run-on experiments have shown that the phenomenon occurs at the post-transcriptional level (Elmayan and Vaucheret, Plant J. 9:787-797, 1996).

It is possible, by inducing a mutation of the transformed plants, not only to eliminate these inhibition phenomena, but also to increase the level of expression of the heterologous genes in this mutated plant (Elmayan et al., 1998, Plant Cell 10:1747-1757, 1998).

It is therefore imagined that it is, today, essential to identify the genes involved in post-transcriptional inactivation in order to improve, firstly, the stability of transgene expression in plants and, secondly, the production of recombinant proteins in plants. The identification of these genes is also of great value because of their role in the resistance of plants to viral infections.

A novel plant gene, named *SGS3*, has now been isolated, which is involved in post-transcriptional inactivation phenomena in transgenic plants, and in the resistance of plants to viral infections. Inhibition of this gene leads to inhibition of the post-transcriptional inactivation phenomena, in particular in transgenic plants comprising a heterologous gene encoding a particular peptide or protein, allowing a particularly high level of expression of said peptide or of said protein. A subject of the invention is also the overexpression of the *SGS3* gene, for preparing plants which are more resistant to viral infections.

Description of the sequence listing

SEQ ID No. 1: *SGS3* gene of *Arabidopsis thaliana*

SEQ ID No. 2: cDNA of the *SGS3* gene of *Arabidopsis thaliana*

SEQ ID No. 3: *SGS3* polypeptide of *Arabidopsis thaliana*

Description of the invention

SGS3 polynucleotides

The present invention relates to *SGS3* polynucleotides, in particular polynucleotides comprising an *SGS3* plant gene. Preferentially, the polynucleotides of the present invention comprise the coding sequence of an *SGS3* plant gene. The *SGS3* gene may be isolated from dicotyledon plants, such as *Arabidopsis*, tobacco, rapeseed, sunflower, soybean,

cotton, clover or duckweed (lemnae), or from monocotyledon plants, such as rice, maize or wheat. Advantageously, the *SGS3* gene is isolated from dicotyledon plants, in particular crucifers such as *Arabidopsis* or rapeseed. Preferably, the polynucleotides of the invention comprise an *SGS3* gene of *Arabidopsis thaliana*.

The term "*SGS3* polynucleotides" denotes all of the polynucleotides of the present invention, preferably the polynucleotides of the genomic sequence of *SGS3*, the polynucleotides of the cDNA sequence of *SGS3*, and also the polynucleotides encoding the *SGS3* polypeptides of the present invention. The term "*SGS3* polynucleotides" also denotes recombinant polynucleotides comprising said polynucleotides.

According to the present invention, the term "polynucleotide" is intended to mean a single-stranded nucleotide chain, or the chain complementary thereto, or a double-stranded nucleotide chain, possibly being of the DNA or RNA type. Preferably, the polynucleotides of the invention are of the DNA type, in particular double-stranded DNA. The term "polynucleotide" also denotes the modified polynucleotides and oligonucleotides.

The polynucleotides of the present invention are isolated or purified from their natural environment. Preferably, the polynucleotides of the present invention may be prepared using the

conventional molecular biology techniques as described by Sambrook et al. (Molecular Cloning: A Laboratory Manual, 1989), or by chemical synthesis.

The invention comprises polynucleotides of the genomic sequence of the *SGS3* gene. This genomic sequence comprises 5 exons (positions 696-1658, 1732-2023, 2135-2379, 2482-2648 and 2739-2949 of SEQ ID No. 1), 4 introns (positions 1659-1731, 2024-2134, 2380-2481 and 2649-2738 of SEQ ID No. 1), and 5' and 3' regulatory sequences. In a preferred embodiment of the invention, the polynucleotides of the genomic sequence of *SGS3* comprise a polynucleotide chosen from the following polynucleotides:

a) the polynucleotide of SEQ ID No. 1,

b) a polynucleotide comprising at least one exon of SEQ ID No. 1,

c) a polynucleotide comprising a combination of exons of SEQ ID No. 1.

The present invention also relates to a polynucleotide comprising a 5' or 3' regulatory sequence of the *SGS3* gene. In a first embodiment, the invention relates to a 5' regulatory polynucleotide comprising the polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1. In a second embodiment, the invention relates to a 3' regulatory polynucleotide comprising the polynucleotide the sequence of which is between position 2950 and position 3275 of SEQ ID No. 1.

A subject of the invention is also a promoter of the *SGS3* gene of *Arabidopsis thaliana*. Preferably, the *SGS3* gene promoter comprises a polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1. In another embodiment of the invention, the *SGS3* gene promoter comprises a biologically active fragment of a polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1.

The term "biologically active fragment" above is intended to mean a polynucleotide having promoter activity, and preferably promoter activity in plants. The techniques which make it possible to establish the promoter activity of a polynucleotide are well known to those skilled in the art. These techniques conventionally involve the use of an expression vector comprising, in the direction of transcription, the polynucleotide to be tested and a reporter gene (see Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989).

The invention also extends to the polynucleotides comprising a polynucleotide chosen from the following polynucleotides:

a) a polynucleotide homologous to a polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1,

b) a polynucleotide capable of selectively hybridizing to a polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1. Preferably, these polynucleotides have promoter activity in plant cells and plants.

The invention also relates to a terminator sequence of the *SGS3* gene of *Arabidopsis thaliana*. Preferably, the *SGS3* gene terminator sequence comprises a polynucleotide the sequence of which is between position 2950 and position 3275 of SEQ ID No. 1. In another embodiment of the invention, the *SGS3* gene terminator sequence comprises a biologically active fragment of a polynucleotide the sequence of which is between position 2950 and position 3275 of SEQ ID No. 1.

The invention also relates to polynucleotides of the *SGS3* cDNA. Preferably, the polynucleotides of the coding sequence of an *SGS3* plant gene comprise polynucleotides of SEQ ID No. 2.

The invention also extends to the polynucleotides comprising a polynucleotide chosen from the following polynucleotides:

a) a polynucleotide homologous to a polynucleotide according to SEQ ID No. 1 or SEQ ID No. 2;

b) a polynucleotide capable of selectively hybridizing to a polynucleotide according to SEQ ID No. 1 or SEQ ID No. 2.

Preferably, the polynucleotides homologous to a reference polynucleotide, or selectively hybridizing to a reference polynucleotide, conserve the function of the reference sequence. The polynucleotides of the present invention preferably encode a polypeptide essential for post-transcriptional inactivation in plants. Preferentially, the polynucleotides of the present invention restore an sgs3 mutant of *Arabidopsis thaliana*. These mutants, and the method for producing them, are described in Elmayan et al. (Plant Cell, 10:1747-1757, 1998). Other methods which make it possible to construct *Arabidopsis thaliana* mutants in which the *SGS3* gene is inactivated are well known to those skilled in the art. The methods for producing *Arabidopsis thaliana* mutants are widely described in the literature.

According to the invention, the term "homolog" is intended to mean a polynucleotide having one or more sequence modifications compared to the reference sequence. These modifications may be deletions, additions or substitutions of one or more nucleotides of the reference sequence. Advantageously, the percentage homology will be at least 70%, 75%, 80%, 85%, 90%, 95%, and preferably at least 98%, and more preferentially at least 99%, compared to the reference sequence. The methods for measuring and identifying homologies between nucleic acid sequences are well known to those skilled in the art. Use may be made, for

example, of the PILEUP or BLAST programs (in particular Altschul et al., J. Mol. Evol., 36:290-300, 1993; Altschul et al., J. Mol. Biol., 215:403-10, 1990). The invention therefore relates to polynucleotides comprising polynucleotides exhibiting at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, and preferably at least 98%, and more preferentially at least 99%, homology with the *SGS3* polynucleotides, the polynucleotides of SEQ ID No. 1 or the polynucleotides of SEQ ID No. 2. Preferably, the invention relates to a polynucleotide comprising a polynucleotide of at least 50, 100, 200, 300, 400, 500, 1000 nucleotides, exhibiting at least 70%, 75%, 80%, 85%, 90%, 95%, 98% and preferably at least 98%, and more preferentially at least 99%, homology with the *SGS3* polynucleotides, the polynucleotides of SEQ ID No. 1 or the polynucleotides of SEQ ID No. 2. Preferably, these homologs conserve the function of the reference sequence.

According to the invention, the expression "sequence capable of selectively hybridizing" is intended to mean the sequences which hybridize with the reference sequence at a level significantly greater than the background noise. The level of the signal generated by the interaction between the sequence capable of selectively hybridizing and the reference sequences is generally 10 times, preferably 100 times, more intense than that of the interaction of the other DNA sequences generating the background noise. The

stringent hybridization conditions which allow selective hybridization are well known to those skilled in the art. In general the temperature for hybridization and for washing is at least 5°C lower than the Tm of the reference sequence at a given pH and for a given ionic strength. Typically, the hybridization temperature is at least 30°C for a polynucleotide of 15 to 50 nucleotides, and at least 60°C for a polynucleotide of more than 50 nucleotides. By way of example, the hybridization is carried out in the following buffer: 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA. The washes are, for example, carried out successively at low stringency in a 2X SSC buffer containing 0.1% SDS, at medium stringency in a 0.5X SSC buffer containing 01% SDS and at high stringency in a 0.1X SSC buffer containing 0.1% SDS. The hybridization may, of course, be carried out according to other normal methods well known to those skilled in the art (in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989). The invention therefore relates to polynucleotides comprising a polynucleotide capable of selectively hybridizing with the polynucleotide of SEQ ID No. 1 or the polynucleotide of SEQ ID No. 2. Preferably, the invention relates to a polynucleotide comprising a polynucleotide of at least 50, 100, 200, 300, 400, 500, 1000 nucleotides, capable of selectively hybridizing

with the polynucleotide of SEQ ID No. 1 or the polynucleotide of SEQ ID No. 2. Preferentially, these polynucleotides selectively hybridizing to a reference polynucleotide conserve the function of the reference sequence.

A subject of the present invention is also antisense polynucleotides which allow inhibition of the expression of an *SGS3* plant gene. The antisense polynucleotides hybridize specifically to the mRNA of an *SGS3* plant gene, thus interfering with the expression of this gene. The techniques for inhibiting the expression of a protein with an antisense polynucleotide are well known to those skilled in the art and widely described in the literature, in particular by Judelson et al. (*Gene*, 133:63-69, 1993) and also by Prokish et al. (Mol. Gen. Genet. 256:104-114, 1997).

The antisense polynucleotides of the present invention hybridize to the mRNA of an *SGS3* plant gene over its entire length, or only to a part of the mRNA of an *SGS3* plant gene. The antisense polynucleotides of the present invention may be completely complementary to the mRNA of an *SGS3* plant gene, or sufficiently homologous to allow pairing and inhibition of the expression of an *SGS3* plant gene.

A subject of the present invention is therefore also polynucleotides comprising an antisense polynucleotide of an *SGS3* plant gene, and

preferentially an antisense polynucleotide of the coding sequence of the *SGS3* gene of SEQ ID No. 2. Preferentially, the antisense polynucleotides of the present invention are derived from a polynucleotide of SEQ ID No. 2. According to a first embodiment, the antisense polynucleotides of the present invention comprise the polynucleotide of SEQ ID No. 2. According to a second embodiment, the antisense polynucleotides of the present invention comprise a fragment of at least 100 nucleotides, preferably of at least 500 nucleotides, and preferentially of at least 1000 nucleotides, of SEQ ID No. 2. According to a third embodiment, the antisense polynucleotides of the present invention comprise a polynucleotide exhibiting at least 85%, 90%, 95%, and preferably at least 98%, and more preferentially at least 99%, homology with a polynucleotide of SEQ ID No. 2. According to another embodiment, the antisense polynucleotides of the present invention comprise a polynucleotide exhibiting at least 85%, 90%, 95%, and preferably at least 98%, and more preferentially at least 99%, homology with a fragment of at least 100 nucleotides, preferably of at least 500 nucleotides, and preferentially of at least 1000 nucleotides, of SEQ ID No. 2.

Preferably, the antisense polynucleotides of the present invention specifically inhibit the expression of an *SGS3* gene in plants.

According to a preferred embodiment, the antisense polynucleotides of the present invention are expressed in plant cells or plants using an expression cassette.

The present invention relates to the use of a polynucleotide, or of a fragment of a polynucleotide, of SEQ ID No. 1 and of SEQ ID No. 2 according to the invention, for identifying the *SGS3* gene in other plants. The cloning is carried out, for example, by screening cDNA libraries or genomic DNA libraries with a polynucleotide, or a fragment of a polynucleotide, of SEQ ID No. 1 and of SEQ ID No. 2. These libraries may also be screened by PCR using specific or degenerate oligonucleotides derived from SEQ ID No. 1 or from SEQ ID No. 2. The techniques for constructing and screening these libraries are well known to those skilled in the art (see in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989). *SGS3* plant genes may also be identified in the databases by nucleotide or protein BLAST using SEQ ID Nos. 1-3.

Preferably, it is verified that the cloned genes carry out the same function as the *SGS3* gene of *Arabidopsis thaliana*, by introducing the genes identified into *SGS3* mutants and by testing for restoration of the post-transcriptional inactivation (see below).

A subject of the invention is also polynucleotides comprising a polynucleotide encoding a polypeptide according to the invention.

SGS3 polypeptides

The present invention also relates to SGS3 polypeptides. The term "SGS3 polypeptides" denotes all of the polypeptides of the present invention, and also the polypeptides for which the polynucleotides of the present invention code. The term "SGS3 polypeptides" also denotes fusion proteins, recombinant proteins or chimeric proteins comprising these polypeptides. In the present description, the term "polypeptide" also denotes proteins and peptides, and also modified polypeptides.

The polypeptides of the invention are isolated or purified from their natural environment. The polypeptides may be prepared by means of various processes. These processes are, in particular, purification from natural sources such as cells naturally expressing these polypeptides, production of recombinant polypeptides by suitable host cells and subsequent purification thereof, production by chemical synthesis or, finally, a combination of these various approaches. The various production processes are well known to those skilled in the art. Thus, the SGS3 polypeptides of the present invention may be isolated from plants expressing SGS3 polypeptides. Preferably,

the SGS3 polypeptides of the present invention are isolated from recombinant host organisms expressing a heterologous SGS3 polypeptide or expressing a natural SGS3 polypeptide under the control of a heterologous promoter. These organisms are preferentially chosen from bacteria, yeasts, fungi, animal cells, plant cells or plants.

A subject of the present invention is a polypeptide of sequence SEQ ID No. 3, and also a polypeptide comprising a polypeptide of sequence SEQ ID No. 3. The invention also comprises polypeptides comprising a fragment or a homolog of an SGS3 polypeptide, and more particularly of the polypeptide of SEQ ID No. 3.

The term "fragment" of a polypeptide denotes a polypeptide comprising part but not all of the polypeptide from which it is derived. The invention relates to a polypeptide comprising a fragment of at least 10, 15, 20, 25, 30, 35, 40, 50 amino acids of a polypeptide of SEQ ID No. 3. Preferably, these fragments conserve at least one biological activity of the polypeptide from which they are derived. Preferentially, this activity relates to post-transcriptional inactivation in plants. Preferably, the polypeptides of the present invention restore an sgs3 mutant of *Arabidopsis thaliana*.

The term "homolog" denotes a polypeptide according to the invention denotes a polypeptide which

may have a deletion, an addition or a substitution of at least one amino acid. A subject of the invention is a polypeptide exhibiting at least 75%, 80%, 85%, 90%, 95%, 98%, and preferentially 99%, of amino acids identical to a polypeptide of SEQ ID No. 3. Preferably, these homologous polypeptides conserve the same biological activity. Preferentially, this activity relates to post-transcriptional inactivation in plants. Preferably, the polypeptides of the present invention restore an sgs3 mutant of *Arabidopsis thaliana*.

Expression cassettes

The *SGS3* gene may be expressed or overexpressed in various host organisms, such as plants. The present invention relates in particular to the overexpression of the *SGS3* gene in plants or plant cells in order to improve their resistance to viruses. The *SGS3* gene may be expressed in a host organism, under the control of the *SGS3* promoter of the present invention or under the control of a heterologous promoter, and preferably under the control of a promoter which is functional in plants. According to one embodiment of the invention, a polynucleotide encoding an SGS3 polypeptide is inserted into an expression cassette using cloning techniques well known to those skilled in the art. This expression cassette comprises the elements required for the transcription and translation of the sequences encoding the SGS3

polypeptide. Advantageously, this expression cassette comprises both elements for making a host cell produce an SGS3 polypeptide and elements required for regulating this expression. In a first embodiment, the expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, an *SGS3* plant gene, or the coding sequence of an *SGS3* plant gene, and a sequence which is a terminator in said host organism. In another embodiment, the expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, a polynucleotide encoding an SGS3 polypeptide and a sequence which is a terminator in said host organism. Preferentially, the expression cassette comprises, in the direction of transcription, a promoter which is functional in a host organism, a polynucleotide chosen from the following polynucleotides:

a) a polynucleotide encoding an SGS3 polypeptide of SEQ ID No. 3, encoding a homolog or encoding a fragment of a polypeptide of SEQ ID No. 3;

b) a polynucleotide of SEQ ID No. 1;

c) a polynucleotide of SEQ ID No. 2;

d) a polynucleotide homologous to a polynucleotide as defined in b) or c);

e) a polynucleotide capable of hybridizing specifically to a polynucleotide as defined in b) or c);

f) a polynucleotide comprising a fragment of a polynucleotide as defined in b), c), d) and e),

and a sequence which is a terminator in said host organism.

In another embodiment, the expression cassettes of the present invention allow the expression of an antisense polynucleotide, for inhibiting the expression of the *SGS3* gene in a plant. For expressing an antisense polynucleotide in a plant, the expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, an antisense polynucleotide of the coding sequence of an *SGS*3 plant gene and a terminator sequence which is functional in said host organism. Preferably, the expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, an antisense polynucleotide of the coding sequence of the *SGS3* gene of SEQ ID No. 2 and a terminator sequence which is functional in said host organism. Preferentially, the antisense polynucleotides of the present invention are expressed under the control of an inducible promoter.

In a preferred embodiment, the subject of the invention is an expression cassette comprising, in the direction of transcription:

a) a promoter which is functional in a host organism; and

b) an SGS3 polynucleotide according to the invention in the antisense orientation; and

c) a sequence which is a terminator in said host organism.

The *SGS3* promoter may be used to express a heterologous gene in a host organism, and in particular in plant cells or in plants. A subject of the invention is therefore also expression cassettes comprising the promoter of an *SGS3* plant gene, functionally combined with a sequence encoding a heterologous protein, allowing the expression of said protein in plant cells or plants. In one embodiment, the expression cassette according to invention comprises, in the direction of transcription, the *SGS3* promoter of *Arabidopsis thaliana*, the coding sequence for the heterologous protein and a terminator sequence which is functional in plant cells and plants. Preferably, the expression cassette according to the invention comprises, in the direction of transcription, a polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1, or a biologically active fragment of the polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1, the

sequence encoding a heterologous polypeptide and a terminator sequence which is functional in plant cells and plants.

The expression cassettes according to the present invention may also include any other sequence required for the expression of the gene of interest, such as, for example regulatory elements or signal sequences allowing the polypeptide of interest to be addressed.

The techniques for constructing these expression cassettes are widely described in the literature (see in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989).

A subject of the present invention is also a polynucleotide comprising an expression cassette according to the invention, and in particular a vector comprising an expression cassette according to the invention.

Advantageously, the expression cassettes according to the present invention are inserted into a vector for replicating them or for transforming a host organism.

Certain elements of the expression cassettes according to the invention are illustrated below in a nonlimiting manner.

Promoters

Any type of promoter sequence may be used in the expression cassettes according to the invention.

The choice of the promoter will depend in particular on the host organism chosen for expressing the gene of interest. The present invention relates more particularly to the transformation of plants. The choice of the promoter used in the expression cassette determines the temporal and spatial expression of the gene of interest. Some promoters allow specific expression in certain tissues of the plant (roots, leaves or seeds for example) or in certain cells of the plant. Some promoters allow constitutive expression whereas other promoters are, on the contrary, inducible. As a regulatory promoter sequence in plants, use may be made of any promoter sequence for a gene which is naturally expressed in plants, in particular a promoter which is expressed in particular in the leaves of plants, such as for example "constitutive" promoters of bacterial, viral or plant origin, or "light-dependent" promoters, such as that of a plant ribulose-biscarboxylase/oxygenase (RuBisCo) small subunit gene, or any suitable known promoter which may be used. Among the promoters of plant origin, mention will be made of the histone promoters as described in application EP 0 507 698, or the rice actin promoter (US 5,641,876). Among the promoters of a plant virus gene, mention will be made of that of the cauliflower mosaic virus (CAMV 19S or 35S), or the promoter of the circovirus (AU 689 311). Use may also be made of a regulatory promoter sequence specific for particular

regions or tissues of plants, and more particularly promoters specific for seeds (Datla et al., Biotechnology Ann. Rev. 3:269-296, 1997). Use may also be made of an inducible promoter advantageously chosen from the phenylalanine ammonia lyase (PAL), HMG-CoA reductase (HMG), chitinase, glucanase, proteinase inhibitor (PI) or PR1 family gene promoters, the nopaline synthase (nos) promoter, the vspB gene promoter (US 5,670,349), the HMG2 promoter (US 5 670 349), the apple beta-galactosidase (ABG1) promoter or the apple aminocyclopropane carboxylate syntase (ACC synthase) promoter (WO 98/45445).

Use may also be made of the *SGS3* gene promoter of *Arabidopsis thaliana*.

Regulatory sequences for expression

In the expression cassettes of the present invention, use may be made of any regulatory sequence which makes it possible to increase the level of expression of the coding sequence inserted into said expression cassette. According to the invention, use may in particular be made, in combination with the regulatory promoter sequence, of other regulatory sequences, which are located between the promoter and the coding sequence, such as transcription activators ("enhancer"). Among the virus-derived leader sequences, mention will be made, for example, of the tobacco mosaic virus (TMV) activator described in application WO 87/07644, or the tobacco etch virus (TEV) activator.

Various plant intron-derived sequences may also be used to increase the level of expression of the gene of interest, in particular in monocotyledon plants. Mention will be made, for example, of intron I of the maize gene, AdhI (Callis et al., Genes Develop., 1:1183-1200, 1987).

Terminator sequences

A large variety of terminator sequences can be used in the expression cassettes according to the invention. These sequences allow transcription termination and polyadenylation of the mRNA. Any terminator sequence which is functional in the host organism selected may be used. For expression in plants, use may in particular be made of the *nos* terminator of *Agrobacterium tumefaciens*, or terminator sequences of plant origin, such as for example the histone terminator (see EP 0 633 317), the CaMV 35 S terminator and the *tml* terminator. These terminator sequences can be used in monocotyledon and dicotyledon plants.

The terminator sequence of the *SGS3* gene of *Arabidopsis thaliana* is another example of a terminator sequence which can be used in the expression cassettes according to the invention.

Heterologous genes

Any gene of interest may be expressed in a host organism under the control of an *SGS3* promoter. Preferably, the *SGS3* promoter is used for expressing a

heterologous gene in plant cells or in a plant. The genes of interest which may be expressed in plants under the control of an *SGS3* promoter are more widely illustrated below.

Vectors

The present invention also relates to transformation vectors or expression vectors comprising at least one *SGS3* polynucleotide or one expression cassette according to the present invention. The vectors of the present invention are in particular used to transform a host organism and to express an SGS3 polypeptide or an *SGS3* polynucleotide, in said host organism. The host organism is, for example, a bacterium, a yeast, a fungus, a plant cell or a plant. This vector may in particular consist of a plasmid, a cosmid, a bacteriophage or a virus, into which an *SGS3* polynucleotide or an expression cassette according to the invention is inserted. In general, any vector capable of being maintained, of self-replicating or of propagating in a host cell in order to induce the expression of a polynucleotide or a polypeptide may be used.

The techniques for constructing these vectors and the techniques for inserting a suitable sequence into these vectors are widely described in the literature (see in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989).

Advantageously, the vectors according to the invention comprise at least one origin of replication. Preferably, the vectors of the invention also comprise at least one selection marker and preferably a selection marker which can be used in plant cells or in plants. Among the selection markers, mention may be made of the genes for resistance to antibiotics, such as the *nptII* gene for canamycin resistance (Bevan et al., Nature 304:184-187, 1983) and the *hph* gene for hygromycin resistance (Gritz et al., Gene 25:179-188, 1983). Mention will also be made of the genes for tolerance to herbicides, such as the *bar* gene (White et al., NAR 18:1062, 1990) for bialaphos tolerance, the EPSPS gene (US 5,188,642) for glyophosate tolerance or the HPPD gene (WO 96/38567) for isoxazole tolerance. Use may also be made of the genes encoding easily identifiable reporter enzymes, such as the GUS enzyme, or genes encoding pigments and enzymes which regulate the production of pigments, in the transformed cells. Such selection marker genes are in particular described in patent applications EP 242 236, EP 242 246, GB 2 197 653, WO 91/02071, WO 95/06128, WO 96/38567 or WO 97/04103.

Advantageously, these vectors are used for transforming a host organism. Those skilled in the art will choose the suitable transformation vectors in particular as a function of the host organism to be

transformed and as a function of the transformation technique used.

For transforming plant cells or plants, it will in particular be a virus which can be used for the transformation of developed plants, and which also contains its own elements for replication and for expression. Preferentially the vector for transforming the plant cells or plants according to the invention is a plasmid.

Many vectors have been developed for transforming plants with *Agrobacterium tumefaciens*. Other vectors are used for the transformation techniques which are not based on the use of *Agrobacterium*. These vectors are well known to those skilled in the art and widely described in the literature.

Transformation

A subject of the invention is also a process for transforming host organisms, in particular plant cells, with an *SGS3* polynucleotide, an expression cassette, a transformation vector or an expression vector according to the invention.

According to the present invention, the transformation of the host organism may be obtained by any suitable known means; the transformation techniques, and in particular the techniques for transforming plants, are fully described in the

specialized literature. For the processes for transforming plant cells and for regenerating plants, mention will be made in particular of the following patents and patent applications: US 4,459,355, US 4,536,475, US 5,464,763, US 5,177,010, US 5,187,073, EP 267 159, EP 604 662, EP 672 752, US 4,945,050, US 5,036,006, US 5,100, 792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520, US 5,510,318, US 5,204,253, US 5,405,765, EP 442 174, EP 486 233, EP 486 234, EP 539 563, EP 674 725, WO 91/02071, WO 95/06128 and WO 99/19497.

Some techniques use *Agrobacterium* in particular for transforming dicotyledons. A series of methods consist in using, as a means for transfer into the plant, a chimeric gene inserted into an *Agrobacterium tumefaciens* Ti plasmid or *Agrobacterium rhizogenes* Ri plasmid. Other methods consist in bombarding cells, protoplasts or tissues with particles to which the DNA sequences are attached. Other methods may also be used, such as microinjection or electroporation, or direct precipitation using PEG.

Those skilled in the art will choose the suitable method depending on the nature of the host organism, in particular of the plant cell or of the plant.

Host organisms

The present invention also relates to a host organism transformed with an SGS3 polynucleotide, an expression cassette or a vector according to the invention.

According to the invention, the term "host organism" is intended to mean in particular any monocellular or multicellular, lower or higher organism, in particular chosen from bacteria, yeasts, fungi or plant cells and plants. Advantageously, the bacteria are chosen from *Escherichia coli*, the yeasts are chosen from *Pichia pastoris* and *Saccharomyces cerevisae*, and the fungi are chosen from *Aspergillus niger*. Preferentially, the host organism is a plant cell or a plant.

According to the invention, the term "plant cell" is intended to mean any cell derived from a plant and which may constitute undifferentiated tissues such as calluses, differentiated tissues such as embryos, parts of plants, plants or seeds.

According to the invention, the term "plant" is intended to mean any differentiated multicellular organism capable of photosynthesis, in particular monocotyledons or dicotyledons, more particularly crop plants, which may or may not be intended for animal or human food, such as maize, wheat, barley, sorghum, rapeseed, soybean, rice, sugar cane, beetroot, tobacco, cotton, clover, duckweed (lemnae), etc.

According to a particular embodiment of the invention, the host organism comprises at least one other heterologous gene encoding a peptide, a polypeptide or a protein of interest. The polynucleotide comprising an *SGS3* polynucleotide according to the invention and the other heterologous gene(s) may have been introduced into the host organism simultaneously by means of the same vector comprising them or by means of several vectors, or sequentially by means of several vectors, or alternatively by crossing several host organisms, each comprising a heterologous gene.

According to the invention, the term "heterologous gene" is intended to mean any gene introduced artificially into the host organism, and more particularly integrated artificially into its genome, the methods allowing this introduction or integration possibly being those described previously, the content of the references cited being incorporated herein by way of reference.

The heterologous gene, other than the *SGS3* polynucleotides according to the invention, may be a gene comprising a coding sequence and the 5' and 3' regulatory elements for said coding sequence, which are not modified compared to the natural gene, reintroduced artificially into the genome of a host organism which may be of the same species as that from which the gene was isolated, or a different species. The heterologous

gene may also be a chimeric gene or an expression cassette comprising a coding sequence of plant, bacterial, fungal, viral or animal origin, under the control of regulatory elements which are functional in the host organism and which are different from those naturally functionally linked to the coding sequence.

A subject of the present invention is also the plants containing transformed cells as defined above, in particular the plants regenerated from the transformed cells and their descendants. The regeneration is obtained using any suitable process, which depends on the nature of the species, as described for example in the references above.

The present invention also relates to the genetically modified plants into the genome of which an *SGS3* polynucleotide or an expression cassette according to the invention are integrated in a manner which is stable and transmissible by sexual reproduction.

The present invention also relates to plants obtained by crossing the regenerated plants above with other plants. It also relates to the seeds of transformed plants.

***sgs3* mutants**

The invention also relates to the *sgs3* mutants in which the *SGS3* gene is inactivated. The inactivation of this gene leads to inhibition of the post-transcriptional inactivation phenomena in these mutants.

The inactivation of the *SGS3* gene in the plants may be obtained by means of various mutagenesis, site-directed mutagenesis or "gene machine" techniques, or using homologous recombination techniques (Kempin, S.A. et al., Targeted disruption in *Arabidopsis*, Nature 389:802-803, 1997). These techniques are well known to those skilled in the art. Among the mutagenesis techniques, mention will be made of the chemical mutagenesis techniques. Mention will also be made of the mutagenesis techniques using transposable elements which allow inactivation of genes by insertion. When the mutagenesis techniques used do not make it possible to specifically inactivate the *SGS3* gene, the mutants obtained are screened in order to identify the mutants affected in the *SGS3* gene. This screening may be phenotypic screening or screening based on the amplification and sequencing of the *SGS3* gene in the mutants, according to techniques described in the literature. Among the site-directed mutagenesis techniques, mention will be made of chimeraplasty (US 6,010,907).

In one particular embodiment of the invention, mutants are obtained according to the process described by Elmayan et al. (Plant Cell, 10:1747-1757, 1998) by treating seeds with a solution of EMS (ethyl methanesulfonate) at 0.4%. The mutants are then analyzed in order to identify the mutants affected in the *SGS3* gene. This screening may, for example, be carried out by PCR.

The present invention also relates to the use of SGS3 mutants for identifying *SGS3* genes in other plant species, such as for example tobacco, rapeseed, sunflower, soybean, cotton, rice, maize, sorghum, barley or wheat. The functional homologs of *SGS3* in other species are identified by complementation of the *sgs3* mutants according to the invention. A polynucleotide which restores the wild-type phenotype of post-transcriptional inactivation is cloned. The sequence of this polynucleotide is then determined in order to identify the constituent elements of the cloned gene.

Inhibition/inactivation of *SGS3* and expression of heterologous genes in plants

The development of genetic transfer techniques has allowed the expression of genes in plants, in particular with a view to improving their agronomic properties or for the production of proteins of interest. However, post-transcriptional inactivation

phenomena constitute a considerable obstacle to the stability of transgene expression in plants. These phenomena of suppression of the expression of the transgene are particularly frequent in the context of strongly expressed transgenes. The present invention relates to a novel plant *SGS3* gene. The inhibition or inactivation of this *SGS3* gene in plants causes inhibition of the post-transcriptional inactivation phenomenon and therefore makes it possible to produce plants in which the expression of heterologous genes is more stable, and also plants in which the level of expression of the heterologous genes is higher.

Inactivation/inhibition of the *SGS3* gene in plants

In a first embodiment, the invention relates to a process for expressing a heterologous gene in a plant, characterized in that it comprises transforming the plant with the heterologous gene and inhibiting the expression of the *SGS3* gene in said plant.

Preferably, the invention relates to a process for expressing a heterologous gene in a plant, characterized in that it comprises the following steps:

a) said plant is transformed with said heterologous gene; and

b) the expression of an SGS3 polynucleotide according to the invention is inhibited in said plant.

Preferentially, the inhibition of the expression of the *SGS3* gene comprises transforming the

plant with a polynucleotide comprising a polynucleotide chosen from the following polynucleotides:

a) an antisense polynucleotide of the coding sequence of an *SGS3* plant gene;

b) an antisense polynucleotide of the coding sequence of the *SGS3* gene of SEQ ID No. 2;

c) an expression cassette comprising, in the direction of transcription, a promoter which is functional in a host organism, a polynucleotide as defined in a) or b) and a terminator sequence which is functional in said host organism.

In another embodiment, the invention relates to a process for expressing a heterologous gene in a plant, characterized in that it comprises transforming the plant with the heterologous gene and inactivating the expression of the *SGS3* gene in said plant.

A subject of the invention is also a process for expressing a heterologous gene in a plant, comprising the following steps:

a) said plant is transformed with said heterologous gene;

b) the expression of an SGS3 polynucleotide according to the invention is inactivated in said plant.

In the context of the present invention, it is clearly understood that the step for inactivating or inhibiting the plant *SGS3* gene and the step for transforming the plant with a heterologous gene may be carried out simultaneously on the same plant or

sequentially, or alternatively by crossing several plants. The processes according to the invention may therefore also comprise steps for regenerating plants, for asexual multiplication or for crossing plants.

Heterologous genes

Various heterologous genes of interest may be expressed in the plants in which the expression of the *SGS3* gene is inhibited or inactivated. Preferably, the heterologous gene encodes peptides, proteins or enzymes. They may be reporter proteins, selection markers, or peptides or proteins of interest which confer novel properties on the host organism, more particularly novel agronomic properties for the transformed plants.

Among the genes which confer novel agronomic properties on the transformed plants, mention may be made of the genes which confer tolerance to certain herbicides, those which confer resistance to certain insects, those which confer tolerance to certain diseases, etc. Such genes are in particular described in patent applications WO 91/02071 and WO 95/06128.

Among the genes which confer tolerance to certain herbicides, mention may be made of the Bar gene which confers bialaphos tolerance, the gene encoding a suitable EPSPS which confers resistance to herbicides having EPSPS as the target, such as glyphosate and its salts (US 4,535,060, US 4,769,061, US 5,094,945, US 4,940,835, US 5,188,642 US 4,971,908, US 5,145,783,

US 5,310,667, US 5,312,910, US 5,627,061, US 5,633,435, FR 2 736 926), the gene encoding glyphosate oxydoreductase (US 5,463,175), or a gene encoding an HPPD which confers tolerance to herbicides having HPPD as the target, such as isoxazoles, in particular isoxafutole (FR 95 06800, FR 95 13570), diketonitriles (EP 496 630, EP 496 631) or triketones, in particular sulcotrione (EP 625 505, EP 625 508, US 5,506,195). Such genes encoding an HPPD conferring tolerance to herbicides having HPPD as the target are described in patent application WO 96/38567.

Among the proteins of interest which confer novel properties of resistance to insects, mention will be made more particularly of the Bt proteins widely described in the literature and well known to those skilled in the art. Mention will also be made of the proteins extracted from bacteria such as Photorabdus (WO 97/17432 & WO 98/08932).

Among these proteins or peptides of interest which confer novel properties of resistance to diseases, mention will be made in particular of chitinases, glucanases and oxalate oxidase, all these proteins and their coding sequences being widely described in the literature, or antibacterial and/or antifungal peptides, in particular peptides of less than 100 amino acids rich in cysteines, such as plant defensins or thionine, and more particularly lytic peptides of many origins comprising one or more

disulfide bridges between the cysteines and regions comprising basic amino acids, in particular the following lytic peptides: androctonin (WO 97/30082 and PCT/FR98/01814, filed on August 18, 1998) or drosomycin (PCT/FR98/01462, filed on July 8, 1998).

According to a particular embodiment of the invention, the protein or peptide of interest is chosen from fungal elicitor peptides, in particular elicitins (Kamoun et al., 1993; Panabières et al., 1995).

Mention may also be made of the genes which modify the constitution of the modified plants, in particular the content and the quality of certain essential fatty acids (EP 666 918) or the content and the quality of the proteins, in particular in the leaves and/or the seeds of said plants. Mention will be made in particular of the genes encoding proteins enriched in sulfur-containing amino acids (Korit et al., Eur. J. Biochem. 195:329-334, 1991; WO 98/20133; WO 97/41239; WO 95/31554; WO 94/20828; WO 92/14822). These proteins enriched in sulfur-containing amino acids will also have the function of trapping and storing excess cysteine and/or methionine, making it possible to avoid the possible problems of toxicity linked to overproduction of these sulfur-containing amino acids by trapping them. Mention may also be made of genes encoding peptides rich in sulfur-containing amino acids and more particularly in cysteines, said peptides also having antibacterial and/or antifungal

activity. Mention will be made more particularly of plant defensins, and also lytic peptides of any origin, and more particularly the following lytic peptides: androctonin (WO 97/30082 and PCT/FR98/01814, filed on August 18, 1998) or drosomycin (PCT/FR98/01462, filed on July 8, 1998).

The host organisms of the present invention may also be used for producing proteins of interest in plants or "molecular farming". Specifically, the invention also relates to transformed plants which make it possible to produce higher levels of expression of heterologous genes. Among the proteins of interest, mention will be made in particular of mammalian peptides and proteins. The production of immunoglobulins (US 5,990,385; US 5,639,947, 5,959,177) and of interferon (US 4,956,282) have, for example, been described in plants.

All the methods or operations described below in the examples are given by way of example and correspond to a choice made from the various methods available to achieve the same result. This choice has no bearing on the quality of the result and, consequently, any suitable method may be used by those skilled in the art to achieve the same result. Most of the methods for engineering DNA fragments are described in "Current Protocols in Molecular Biology" Volumes 1 and 2, Ausubel F.M. et al. or in Sambrook et al. 1989.

Description of the figures

FIGURE 1: *sgs3* Mutants

Examples

Example 1

Isolation and identification of the *SGS3* gene of *Arabidopsis*

The SGS3 mutant (affected in the *SGS3* plant gene) was obtained using the same experimental protocol as that which allowed the isolation of the *sgs1* and *sgs2* mutants (Elmayan et al., Plant Cell 10:1747-1757, 1998). The starting line was the L1 line. L1 is a transgenic line obtained by transforming plants of the Columbia ecotype with the 23b construct (Elmayan and Vaucheret, Plant J. 9:787-797, 1996). The L1 line comprises only a single transgenic locus. The glucuronidase activity in the L1 line is 4000 mmol of 4-methylumbelliferone per minute and per microgram of total proteins in the first days of development. This activity then decreases very rapidly to become less than 5 nmol of 4-methylumbelliferone per minute and per microgram of total proteins 11 days after germination. The inactivation of the expression of the 35S-*uidA* transgene is post-transcriptional, as demonstrated in the run-on experiments revealing strong transcription of the 35S-*uidA* transgene in the L1 plants showing very

low GUS activity (Elmayan et al., Plant Cell 10:1747-1757, 1998). For producing mutant plants of the L1 line, 3000 seeds of the L1 line were soaked for 16 hours in a solution of EMS (ethyl methanesulfonate) at 0.4%. The seeds were then sown and the plants produced were cultivated under glass until self-fertilization seeds were produced. These seeds were again sown under glass and, in the plants produced, the GUS activity was measured 1 month after germination. The plants exhibiting high activity at this stage were crossed with plants of the Columbia ecotype (to verify that the transgenic locus remains sensitive to post-transcriptional inactivation), backcrossed with the L1 line (to evaluate the state of recessiveness vs dominance of the mutations produced) and crossed with one another (to classify the various mutants produced into complementation groups, each group defining a gene). 6 independent *sgs3* mutants were thus isolated. These 6 mutations are recessive. The GUS activity in these 6 mutant lines, one month after germination, is between 2500 and 3500 nmol of 4-methylumbelliferone per minute and per microgram of total proteins. The GUS activity in these mutant lines, 1 month after germination, is between 2500 and 3500 nmol of 4-methylumbelliferone per minute and per microgram of total proteins. In order to confirm that the *sgs3* mutations affect the expression of the 35S-*GUS* transgene at the transcriptional level, the GUS

activity, the mRNA accumulation and the transcription rate were measured by fluorimetric tests, by mRNA blot analysis and by "run-on" experiments. The GUS activity is multiplied by a factor of 300 in the *sgs2* mutants compared to the L1 line, while the mRNA accumulation is multiplied by a factor of 250. The transcription rate is only multiplied by a factor of 2.6 compared to the L1 line. In order to verify that the *sgs33* mutations protected against the post-transcriptional inactivation of a gene other than the *uidA* gene, one of the *sgs3* mutants (named *sgs3-2*) was crossed with the 2a3 line (Elmayan et al., Plant Cell 10:1747-1757, 1998). The 2a3 line is a transgenic *Arabidopsis thaliana* line which results from the transformation of a plant of the Columbia ecotype with the 2a construct (Elmayan et al., Plant Cell 10:1747-1757, 1998) containing the transcribed portion of the *Arabidopsis NIA2* gene encoding nitrate reductase under the control of the 35S promoter and the hygromycin resistance gene *hpt*. All the plants of the 2a3 line which are homozygous for the 2a construct exhibit post-transcriptional inactivation of the *Nia2* genes (transgenic and endogenous), leading to chlorosis of the plant and then to its death. When the transgenic 2a3 locus is in the heterozygous state, only some of the plants undergo the post-transcriptional inactivation. The stage at which this inactivation occurs is variable from one plant to the other. In some plants, the inactivation is sufficiently

late to allow the production of pollen and of seeds. The hybrid plants derived from the cross between the *sgs3-2* mutant and the 2a3 line were cultivated under glass and the self-fertilization seeds were harvested. The seeds were sown under glass and the plants produced which exhibited no chlorosis were kept in order to harvest their self-fertilization seeds. We then sowed the various batches of seeds on an agar medium containing 20 mg/l of hygromycin. Among these, some gave only plants which were resistant to hygromycin and showed no sign of chlorosis throughout their development. Among these lines of resistance to the post-transcriptional inactivation of the nitrate reductase genes, some were also homozygous for the 23b construct. We also showed that the plants of all these lines exhibited high GUS activity throughout their development. These results therefore show that the *sgs3* mutation not only protects against the post-transcriptional inactivation of the 35S-*uidA* transgene, but also of the *NIA2* endogenous genes and transgenes. Some of these lines resistant to the post-transcriptional inactivation of the nitrate reductase genes and homozygous for the 2a3 locus no longer contained the 23b construct. These plants were named SGS3-2 2a3.

In order to determine the biological role of the gene corresponding to the *sgs3* mutations, *sgs3-1* mutants were inoculated with the cucumber mosaic virus

(CMV) strain I17F. On the wild-type plants, infection with this viral strain produces plants in which development is slower and modified: smaller leaves of the rosette, long but very flexible floral scape, fertility decreased but not zero. In the *sgs3-1* mutants, infection with this viral strain produces an increased modification of development: the plants have a particularly bushy habit, the leaves of the rosette are small and tendrilled, the floral scape reaches, at the end of development, a size of about 5 cm, and the plants are completely sterile. These experiments therefore show that the gene corresponding to the *sgs3* mutations makes it possible to limit the negative effects on development caused by CMV virus infection.

Two mutants, *sgs3-1* and *sgs3-2*, were crossed with plants of the Landsberg ecotype. From these hybrid (F1) plants resulting from these crosses, the self-fertilization seeds were harvested. These seeds were sown in vitro on an agar medium containing 50 mg/l of canamycin in order to select the plants (F2) containing the 23b transgene. These canamycin-resistant plants were planted out and cultivated under glass. The GUS activity in these plants was measured at various stages of their development. Only the plants exhibiting high GUS activity throughout development (and therefore homozygous for the *sgs3* mutation) were kept and the self-fertilization seeds were harvested. 120 F2 lines homozygous for the *sgs3-1* mutation (F2-1 lines) and 90

F2 lines homozygous for the *sgs3-2* mutation (F2-2 lines) were thus produced. The self-fertilization seeds from each of these lines were sown under glass and, for each line, a pool of plants was harvested in order to extract the DNA therefrom. These DNAs were used to locate the *sgs3* mutations on the *Arabidopsis* genome. The initial locating was carried out using the F2-1 lines. The F2-2 lines then allowed us to verify that the *sgs3-2* mutation was located in the same region of the genome as the *sgs3-1* mutation. These analyses show that the *sgs3* mutations were located between the 13H2L and 3B3D molecular markers. The polymorphism corresponding to the 13H2L molecular marker was revealed by hybridization (of the Southern blot type) of the total DNA of *Arabidopsis* plants, digested with the HindIII restriction enzyme, with a radioactive DNA fragment corresponding to the left end of the 13H2 yeast artificial chromosome (YAC) (13H2L probe). The polymorphism corresponding to the 3B3D molecular marker was revealed by hybridization (of the Southern blot type) of the total DNA of Arabidopsis plants, digested with the HindIII restriction enzyme, with a radioactive DNA fragment corresponding to the right end of the 3B3 YAC (3B3 probe).

We were able to determine, by molecular hybridization of the Southern type, on a membrane onto which the DNA of a bacteria artificial chromosome library (BAC IGF) was transferred, with the radioactive

DNA fragment corresponding to the 13H2L and 3B3D probes, that these 2 DNA fragments hybridized on the same BAC: BAC F20I20. These results therefore show that the *sgs3-1* and *sgs3-2* mutations affect a DNA sequence included in the BAC F20I20.

DNA from the BAC F20I20 was then purified. It was partially digested with the Sau3AI restriction enzyme. The resulting DNA fragments were cloned at the BamHI site of the transfer DNA of the binary plasmid (allowing the transformation of plants via *Agrobacterium*) pBin+. The resulting plasmids were introduced into E. coli and then into the *Agrobacterium tumefaciens* strain C58pMP90. The resulting bacterial strains were used to transform plants of the *sgs3-2* 2a3 lines. The bacterial strain 356 made it possible to produce 20 transgenic lines. Among these 20 lines, 19 showed signs of chlorosis identical to those observed on the 2a3 line. On 3 of these plants, we were able to show, by hybridization of the northern type using the *Arabidopsis thaliana NIA2* gene as the probe, that this chlorosis resulted from the non-accumulation of the transcripts of the nitrate reductase genes (transgenic and endogenous) and was therefore due to the post-transcriptional inactivation of the nitrate reductase genes. Among these 19 plants, 2 gave self-fertilization seeds. The plants derived from these seeds, cultivated under glass, also showed signs of chlorosis.

The DNA sequence which was inserted at the BamHI site of the pBin+ plasmid and which had led to the isolation of the bacterial strain 356 was determined. Subclones of the 356 clone were produced in the pBin+ vector and the same *sgs3-2* 2a3 line was transformed with these subclones in order to determine those capable of restoring the function of the *SGS3* gene. The smallest subclone capable of restoring this function constitutes the *SGS3* gene such as it is described in this patent. It was possible to predict the ORF of *SGS3* by computer analysis. The sequence of the cDNA containing the ORF of the *SGS3* gene, and therefore the position of the promoter, terminator and intronic sequences of *SGS3*, were verified after having isolated and cloned this sequence. In order to isolate, we first performed a reverse-transcription reaction using *Arabidopsis thaliana* total RNA. We then performed a PCR reaction on this pool of cDNA using the pair of primers p356AD' (AAAATGAGTTCTAGGGCTGGTCC) and P356Y' (GTCTCAATCATCTTCATTGTGAAGGCC). These primers are located at the 2 ends of the ORF of *SGS3*. This PCR product was cloned and sequenced.

Using the BLAST program, no significant homology could be found between the *SGS3* sequence (nucleotide or amino acid) and any sequence present in the data bases.

Example 2

Analysis of the *sgs3* mutants

The sequence of the *SGS3* gene was determined in 5 *sgs3* mutants. A PCR reaction was carried out on the genomic DNA of these 5 mutants using the p356AD' and P356Y' primers (see Example 1). This reaction made it possible to amplify the entire *SGS3* gene. The fragment amplified by this PCR reaction was sequenced.

Five distinct point mutations were thus identified in the various *sgs3* mutants. These mutations correspond to four stop codons and one amino acid change. The various mutations observed in the *sgs3* mutants are represented in Figure 1. The amino acid marked in bold indicates the position of the mutation in the SGS3 polypeptide. * indicates the presence of a stop codon and () indicates a new amino acid substituted for the amino acid marked in bold affected by the mutation.

Example 3

Construction of expression cassettes for the overexpression and inhibition of *SGS3*

A PCR-type reaction is first carried out on *Arabidopsis thaliana* complementary DNA using, as primers, the following oligonucleotides:

p356AD' : AAAATGAGTTCTAGGGCTGGTCC

P356Y' : GTCTCAATCATCTTCATTGTGAAGGCC

The nucleotide sequence thus obtained is then treated with the "klenow" enzyme in order to generate "blunt" ends at the ends of the amplified sequence. The sequence is then cloned between the 35S promoter and the terminator of the cauliflower mosaic virus, at the SmaI site of the pRT100 vector.

For the overexpression of *SGS3*, the clones are selected such that the sequence corresponding to p356AD' is located close to the 35S promoter. For the inhibition of *SGS3*, the clones are selected such that the sequence corresponding to p356Y' is located close to the 35S promoter. This Assgs3 construct allows the expression of an antisense mRNA for the *SGS3* mRNA.

Example 4

Transformation of plants

The expression cassettes constructed as described above are then introduced into a binary vector so as to allow their introduction, via *Agrobacterium tumefaciens* into plants. The binary vector used is the pBIN+ plasmid (Van Engelen et al., Transgenic Research 4, 288-290, 1995). This is performed by digesting the constructs obtained above, with the SphI enzyme (which releases the expression cassettes), and ligating the product of this digestion to the pBIN+ plasmid digested with the SphI enzyme.

Example 5

Inhibition of expression of the *SGS3* plant gene with antisense sequences

The complete cDNA of the *SGS3* plant gene was cloned, in the antisense orientation (aSGS3), between the 35S promoter (p35S) and the 35S terminator (t35S). The chimeric gene p35S-aSGS3-t35S was re-cloned into the pBiB-Hyg binary vector and then transferred into *Agrobacterium tumefaciens*. Plants of the L1 line (p35S-GUS-tRbcS gene subjected to PTGS) were transformed by soaking in agrobacteria. The transformed plants were selected on medium supplemented with hygromycin. The GUS activity of the p35S-GUS-tRbcS transgene was measured in the nontransformed L1 plants, in 28 hygromycin-resistant transformants and also in the sgs3 mutants obtained by EMS mutagenesis of the L1 line. The GUS activity in the nontransformed L1 plants is between 0 and 10 nmol MU/min/µg of proteins, while the GUS activity in the sgs3 mutants is between 3000 and 5500 nmol MU/min/µg of proteins. 11 of the 28 hygromycin-resistant transformants showed a GUS activity of between 3000 and 5500 nmol MU/min/µg of proteins, showing that the *SGS3* plant gene may be inhibited by the chimeric gene p35S-aSGS3-t35S, thus mimicking an sgs3 mutation.

Claims

1. A polynucleotide, characterized in that it comprises a polynucleotide chosen from the following polynucleotides:

a) the polynucleotide of SEQ ID No. 1, and

b) the polynucleotide of SEQ ID No. 2.

2. A polynucleotide, characterized in that it comprises a polynucleotide chosen from the following polynucleotides:

a) a polynucleotide capable of selectively hybridizing to a polynucleotide as claimed in claim 1, and

b) a polynucleotide at least 80% homologous to a polynucleotide as claimed in claim 1.

3. The polynucleotide as claimed in claim 2, characterized in that it restores an sgs3 mutant of *Arabidopsis thaliana*.

4. A polynucleotide, characterized in that it comprises the polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1.

5. A polynucleotide, characterized in that it comprises a polynucleotide chosen from the following polynucleotides:

a) a polynucleotide capable of selectively hybridizing to a polynucleotide as claimed in claim 4, and

b) a polynucleotide at least 80% homologous to a polynucleotide as claimed in claim 4.

6. The polynucleotide as claimed in claim 5, characterized in that it has promoter activity in plant cells and plants.

7. A polypeptide, characterized in that it comprises the polypeptide of SEQ ID No. 3.

8. A polypeptide, characterized in that it comprises a polypeptide chosen from the following polypeptides:

a) a biologically active fragment of the polypeptide of SEQ ID No. 3; and

b) a polypeptide at least 80% homologous to the polypeptide of SEQ ID No. 3.

9. The polypeptide as claimed in claim 8, characterized in that it restores an sgs3 mutant of *Arabidopsis thaliana*.

10. A polynucleotide, characterized in that it comprises a polynucleotide encoding a polypeptide as claimed in one of claims 7-9.

11. An expression cassette, characterized in that it comprises, in the direction of transcription:

a) a promoter which is functional in a host organism; and

b) a polynucleotide as claimed in one of claims 1-3 and 10; and

c) a sequence which is a terminator in said host organism.

12. An expression cassette, characterized in that it comprises, in the direction of transcription:

d) a promoter which is functional in a host organism; and

e) a polynucleotide as claimed in one of claims 1-3 and 10, in the antisense orientation; and

f) a sequence which is a terminator in said host organism.

13. An expression cassette, characterized in that it comprises, in the direction of transcription:

a) a polynucleotide as claimed in one of claims 4-6;

b) a polynucleotide encoding a heterologous polypeptide;

c) a sequence which is a terminator in plant cells or plants.

14. An expression vector or transformation vector, comprising a polynucleotide as claimed in one of claims 1-6 and 10 or an expression cassette as claimed in one of claims 11-13.

15. A process for transforming host organisms, in particular plant cells or plants, by integrating into said host organism at least one polynucleotide as claimed in one of claims 1-6 and 10 and/or at least one expression cassette as claimed in one of claims 11-13 and/or at least one vector as claimed in claim 14.

16. A process for expressing a heterologous gene in a plant, characterized in that it comprises the following steps:

a) said plant is transformed with said heterologous gene; and

b) the expression of a polynucleotide as claimed in one of claims 1-3 and 10 is inhibited in said plant.

17. The process as claimed in claim 16, characterized in that step b) comprises transforming said plant with an expression cassette as claimed in claim 12.

18. A process for expressing a heterologous gene in a plant, characterized in that it comprises the following steps:

b) said plant is transformed with said heterologous gene;

c) the expression of a polynucleotide as claimed in one of claims 1-3 and 10 is inactivated in said plant.

19. A transformed host organism comprising at least one polynucleotide as claimed in one of claims 1-6 and 10 and/or of at least one expression cassette as claimed in one of claims 11-13 and/or of at least one vector as claimed in claim 14.

20. The host organism as claimed in claim 19, characterized in that it comprises at least one

heterologous gene encoding a peptide or a protein of interest.

21. The host organism as claimed in either of claims 19 and 20, characterized in that the host organism is chosen from bacteria, yeasts, fungi, plant cells or plants.

22. The host organism as claimed in claim 21, characterized in that the plants are chosen from maize, wheat, barley, sorghum, rapeseed, soybean, rice, beetroot, tobacco and cotton.

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WO 01/05951 A2

(54) Title: NOVEL SGS3 PLANT GENE AND USE THEREOF

(54) Titre: NOUVEAU GENE *SGS3* DE PLANTE ET SON UTILISATION

(57) Abstract: The invention concerns novel polynucleotides comprising the SGS3 plant gene involved in post-transciptional inactivation phenomena in transgenic plants and in the resistance of plants to viral infections, and it use for preparing genetically modified plants.

(57) Abrégé: La présente invention concerne de nouveaux polynucléotides comprenant le gène *SGS3* de plante impliqué dans les phénomènes d'inactivation post-transcriptionnelle dans les plantes transgéniques et dans la résistance des plantes aux infections virales, et son utilisation pour la préparation de plantes génétiquement modifiées.

COMBINED DECLARATION AND POWER OF ATTORNEY

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL SGS3 PLANT GENE AND USES THEREOF

This declaration is of the following type:

- [] original
- [] design
- [X] national stage of PCT/FR00/02052
- [] divisional
- [] continuation
- [] continuation-in-part (C-I-P)

the specification of which: (complete (a), (b), or (c))

(a) [ ] is attached hereto.
(b) [X] was filed on January 11, 2002 as Application Serial No. 10/030,829 and was amended on (if applicable).
(c) [ ] was described and claimed in PCT International Application No. filed on and was amended on (if applicable).

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

[] In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e)

(d) [] no such applications have been filed.
(e) [X] such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
COUNTRY	APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
				[] YES NO []
				[] YES NO []
				[] YES NO []
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				[] YES NO []

Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

Claim for Benefit of Earlier U.S./PCT Application(s) under 35 U.S.C. 120

(complete this part only if this is a divisional, continuation or C-I-P application)

(Application Serial No.)	(Filing Date)	Status (patented, pending, abandoned)

Power of Attorney

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BAKER BOTTS L.L.P. 30 ROCKEFELLER PLAZA, NEW YORK, N.Y. 10112 CUSTOMER NUMBER: 21003	BAKER BOTTS L.L.P. (212) 705-5000

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	LAST NAME Beclin	FIRST NAME Christophe	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY St-Rémy-les-Chevreuses	STATE or FOREIGN COUNTRY France FRX	COUNTRY OF CITIZENSHIP France	
POST OFFICE ADDRESS	POST OFFICE ADDRESS 57, rue du Port Royal	CITY St-Remy-les-Chevreuses	STATE or COUNTRY France	ZIP CODE F-78470
DATE 02/15/2002	SIGNATURE OF INVENTOR			
FULL NAME OF SECOND JOINT INVENTOR, IF ANY	LAST NAME Elmayan	FIRST NAME Taline	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY Dijon	STATE or FOREIGN COUNTRY France FRX	COUNTRY OF CITIZENSHIP France	
POST OFFICE ADDRESS	POST OFFICE ADDRESS 8, ~~rue Pierre Palliot,~~ rue Tour Fondoir	CITY Dijon	STATE or COUNTRY France	ZIP CODE F-21000
DATE 06/03/2002	SIGNATURE OF INVENTOR			
FULL NAME OF THIRD JOINT INVENTOR, IF ANY	LAST NAME Vaucheret	FIRST NAME Hervé	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY ~~Rantzwiller~~ Montigny-le-Bx	STATE or FOREIGN COUNTRY France FRX	COUNTRY OF CITIZENSHIP France	
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DATE 11/03/2002	SIGNATURE OF INVENTOR			
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
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FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
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DATE	SIGNATURE OF INVENTOR			

Check proper box(es) for any added page(s) forming a part of this declaration

[ ] Signature for ninth and subsequent joint inventors. Number of pages added :___.
[ ] Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added ___.
[ ] Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47. Number of pages added __.

<110> Beclin, Christophe
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ggt tat agg cct gag gtt gaa cag ttg gtt caa ggt ttg gca ggg acg   96
Gly Tyr Arg Pro Glu Val Glu Gln Leu Val Gln Gly Leu Ala Gly Thr
             20                  25                  30

aga ctg gct tct tca caa gat gat gga gga gag tgg gag gtc att tcc   144
Arg Leu Ala Ser Ser Gln Asp Asp Gly Gly Glu Trp Glu Val Ile Ser
         35                  40                  45

aag aag aac aag aac aaa cca gga aac act tct gga aaa act tgg gtt   192
Lys Lys Asn Lys Asn Lys Pro Gly Asn Thr Ser Gly Lys Thr Trp Val
     50                  55                  60

tct cag aat tcg aat cct cct aga gct tgg ggt ggt cag cag caa ggg   240
Ser Gln Asn Ser Asn Pro Pro Arg Ala Trp Gly Gly Gln Gln Gln Gly
 65                  70                  75                  80

aga ggt agc aac gta tct ggg aga gga aac aat gta tcc ggg aga ggt   288
Arg Gly Ser Asn Val Ser Gly Arg Gly Asn Asn Val Ser Gly Arg Gly
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                    85                  90                  95

aac ggc aat ggt cgg ggc att caa gct aac ata tct ggt cgg gga cga   336
Asn Gly Asn Gly Arg Gly Ile Gln Ala Asn Ile Ser Gly Arg Gly Arg
            100                 105                 110

gcg ttg agc aga aag tat gat aac aac ttt gtg gca ccc cca cct gta   384
Ala Leu Ser Arg Lys Tyr Asp Asn Asn Phe Val Ala Pro Pro Pro Val
        115                 120                 125

tct cgc cct cct ttg gaa gga gga tgg aat tgg cag gca aga gga ggt   432
Ser Arg Pro Pro Leu Glu Gly Gly Trp Asn Trp Gln Ala Arg Gly Gly
    130                 135                 140

tct gct cag cac aca gct gtg cag gag ttt cct gac gtg gag gat gat   480
Ser Ala Gln His Thr Ala Val Gln Glu Phe Pro Asp Val Glu Asp Asp
145                 150                 155                 160

gtg gat aat gct tct gag gaa gag aat gat tcc gat gct ttg gat gat   528
Val Asp Asn Ala Ser Glu Glu Glu Asn Asp Ser Asp Ala Leu Asp Asp
                165                 170                 175

tct gat gac gac ctt gca agt gat gat tat gac tcg gat gtg agt caa   576
Ser Asp Asp Asp Leu Ala Ser Asp Asp Tyr Asp Ser Asp Val Ser Gln
            180                 185                 190

aag agc cat gga tca cga aag cag aat aag tgg ttc aaa aag ttc ttt   624
Lys Ser His Gly Ser Arg Lys Gln Asn Lys Trp Phe Lys Lys Phe Phe
        195                 200                 205

ggc agc ttg gat agc ttg tcg atc gag cag ata aat gaa cca cag agg   672
Gly Ser Leu Asp Ser Leu Ser Ile Glu Gln Ile Asn Glu Pro Gln Arg
    210                 215                 220

cag tgg cat tgt cca gct tgt cag aac gga cct ggt gcc atc gat tgg   720
Gln Trp His Cys Pro Ala Cys Gln Asn Gly Pro Gly Ala Ile Asp Trp
225                 230                 235                 240

tat aac ctg cac cct cta cta gct cat gcg agg aca aaa gga gct agg   768
Tyr Asn Leu His Pro Leu Leu Ala His Ala Arg Thr Lys Gly Ala Arg
                245                 250                 255

cga gtt aag ctc cat aga gaa ttg gct gaa gtt tta gaa aag gat cta   816
Arg Val Lys Leu His Arg Glu Leu Ala Glu Val Leu Glu Lys Asp Leu
            260                 265                 270

cag atg aga ggc gca tct gtc att cct tgt ggt gag att tat ggg cag   864
Gln Met Arg Gly Ala Ser Val Ile Pro Cys Gly Glu Ile Tyr Gly Gln
        275                 280                 285

tgg aag ggt ttg ggt gag gat gaa aag gat tat gaa att gtc tgg cct   912
Trp Lys Gly Leu Gly Glu Asp Glu Lys Asp Tyr Glu Ile Val Trp Pro
    290                 295                 300

cca atg gtc atc atc atg aat act aga ctg gat aag gac gat aac gat   960
Pro Met Val Ile Ile Met Asn Thr Arg Leu Asp Lys Asp Asp Asn Asp
305                 310                 315                 320

aag tgg ctc ggc atg ggc aac caa gag ctg ctg gaa tac ttc gac aag   1008
Lys Trp Leu Gly Met Gly Asn Gln Glu Leu Leu Glu Tyr Phe Asp Lys
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tat gag gct ctt aga gca cgc cat tcc tat ggt cca cag ggc cat cgt   1056
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Tyr Glu Ala Leu Arg Ala Arg His Ser Tyr Gly Pro Gln Gly His Arg
            340                 345                 350

ggg atg agt gtt ctg atg ttt gag agc agt gcc act ggc tat ttg gag   1104
Gly Met Ser Val Leu Met Phe Glu Ser Ser Ala Thr Gly Tyr Leu Glu
        355                 360                 365

gcc gaa cgc ctc cac cgg gag tta gct gag atg ggg tta gat aga att   1152
Ala Glu Arg Leu His Arg Glu Leu Ala Glu Met Gly Leu Asp Arg Ile
    370                 375                 380

gcc tgg ggt cag aag cgc agt atg ttt tct gga ggt gtt cgc caa ctg   1200
Ala Trp Gly Gln Lys Arg Ser Met Phe Ser Gly Gly Val Arg Gln Leu
385                 390                 395                 400

tat ggc ttc ctt gca acg aag caa gat ctg gac ata ttc aat caa cac   1248
Tyr Gly Phe Leu Ala Thr Lys Gln Asp Leu Asp Ile Phe Asn Gln His
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tct caa ggc aaa aca agg ctg aaa ttc gag ttg aaa tca tac caa gag   1296
Ser Gln Gly Lys Thr Arg Leu Lys Phe Glu Leu Lys Ser Tyr Gln Glu
            420                 425                 430

atg gtt gta aag gag ctg agg cag atc tct gag gac aat cag cag ctg   1344
Met Val Val Lys Glu Leu Arg Gln Ile Ser Glu Asp Asn Gln Gln Leu
        435                 440                 445

aac tac ttt aag aac aag ctc tca aaa cag aac aag cac gcc aag gtg   1392
Asn Tyr Phe Lys Asn Lys Leu Ser Lys Gln Asn Lys His Ala Lys Val
    450                 455                 460

ctt gag gaa tct ctg gaa att atg agc gag aag ctg cgt aga act gca   1440
Leu Glu Glu Ser Leu Glu Ile Met Ser Glu Lys Leu Arg Arg Thr Ala
465                 470                 475                 480

gag gat aat cgg atc gtg aga cag aga act aag atg cag cat gaa cag   1488
Glu Asp Asn Arg Ile Val Arg Gln Arg Thr Lys Met Gln His Glu Gln
                485                 490                 495

aac agg gaa gag atg gat gca cac gac agg ttt ttc atg gat tca atc   1536
Asn Arg Glu Glu Met Asp Ala His Asp Arg Phe Phe Met Asp Ser Ile
            500                 505                 510

aaa cag atc cat gaa aga aga gac gca aag gag gag aat ttc gag atg   1584
Lys Gln Ile His Glu Arg Arg Asp Ala Lys Glu Glu Asn Phe Glu Met
        515                 520                 525

ttg cag cag cag gaa cgt gcc aag gtt gtt ggc cag cag cag cag aac   1632
Leu Gln Gln Gln Glu Arg Ala Lys Val Val Gly Gln Gln Gln Gln Asn
    530                 535                 540

att aat ccc tct agc aat gac gat tgc cga aag aga gct gag gaa gtg   1680
Ile Asn Pro Ser Ser Asn Asp Asp Cys Arg Lys Arg Ala Glu Glu Val
545                 550                 555                 560

tca agc ttc atc gag ttt caa gag aaa gag atg gag gag ttt gtg gaa   1728
Ser Ser Phe Ile Glu Phe Gln Glu Lys Glu Met Glu Glu Phe Val Glu
                565                 570                 575

gag agg gag atg ctg ata aaa gat caa gag aag aag atg gaa gac atg   1776
Glu Arg Glu Met Leu Ile Lys Asp Gln Glu Lys Lys Met Glu Asp Met
            580                 585                 590
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aag aag agg cat cac gag gag ata ttt gat ctg gag aaa gaa ttt gat   1824
Lys Lys Arg His His Glu Glu Ile Phe Asp Leu Glu Lys Glu Phe Asp
        595                 600                 605

gag gct ttg gaa cag ctc atg tac aag cat ggc ctt cac aat gaa gat   1872
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gat tga                                                           1878
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Arg Leu Ala Ser Ser Gln Asp Asp Gly Gly Glu Trp Glu Val Ile Ser
        35                  40                  45
Lys Lys Asn Lys Asn Lys Pro Gly Asn Thr Ser Gly Lys Thr Trp Val
    50                  55                  60
Ser Gln Asn Ser Asn Pro Pro Arg Ala Trp Gly Gly Gln Gln Gln Gly
65                  70                  75                  80
Arg Gly Ser Asn Val Ser Gly Arg Gly Asn Asn Val Ser Gly Arg Gly
                85                  90                  95
Asn Gly Asn Gly Arg Gly Ile Gln Ala Asn Ile Ser Gly Arg Gly Arg
            100                 105                 110
Ala Leu Ser Arg Lys Tyr Asp Asn Asn Phe Val Ala Pro Pro Pro Val
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Ser Arg Pro Pro Leu Glu Gly Gly Trp Asn Trp Gln Ala Arg Gly Gly
    130                 135                 140
Ser Ala Gln His Thr Ala Val Gln Glu Phe Pro Asp Val Glu Asp Asp
145                 150                 155                 160
Val Asp Asn Ala Ser Glu Glu Glu Asn Asp Ser Asp Ala Leu Asp Asp
                165                 170                 175
Ser Asp Asp Asp Leu Ala Ser Asp Asp Tyr Asp Ser Asp Val Ser Gln
            180                 185                 190
Lys Ser His Gly Ser Arg Lys Gln Asn Lys Trp Phe Lys Lys Phe Phe
        195                 200                 205
Gly Ser Leu Asp Ser Leu Ser Ile Glu Gln Ile Asn Glu Pro Gln Arg
    210                 215                 220
Gln Trp His Cys Pro Ala Cys Gln Asn Gly Pro Gly Ala Ile Asp Trp
225                 230                 235                 240
Tyr Asn Leu His Pro Leu Leu Ala His Ala Arg Thr Lys Gly Ala Arg
                245                 250                 255
Arg Val Lys Leu His Arg Glu Leu Ala Glu Val Leu Glu Lys Asp Leu
            260                 265                 270
Gln Met Arg Gly Ala Ser Val Ile Pro Cys Gly Glu Ile Tyr Gly Gln
        275                 280                 285
Trp Lys Gly Leu Gly Glu Asp Glu Lys Asp Tyr Glu Ile Val Trp Pro
    290                 295                 300
Pro Met Val Ile Ile Met Asn Thr Arg Leu Asp Lys Asp Asp Asn Asp
305                 310                 315                 320
Lys Trp Leu Gly Met Gly Asn Gln Glu Leu Leu Glu Tyr Phe Asp Lys
                325                 330                 335
Tyr Glu Ala Leu Arg Ala Arg His Ser Tyr Gly Pro Gln Gly His Arg
            340                 345                 350
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Gly Met Ser Val Leu Met Phe Glu Ser Ser Ala Thr Gly Tyr Leu Glu
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Ala Glu Arg Leu His Arg Glu Leu Ala Glu Met Gly Leu Asp Arg Ile
370 375 380
Ala Trp Gly Gln Lys Arg Ser Met Phe Ser Gly Gly Val Arg Gln Leu
385 390 395 400
Tyr Gly Phe Leu Ala Thr Lys Gln Asp Leu Asp Ile Phe Asn Gln His
405 410 415
Ser Gln Gly Lys Thr Arg Leu Lys Phe Glu Leu Lys Ser Tyr Gln Glu
420 425 430
Met Val Val Lys Glu Leu Arg Gln Ile Ser Glu Asp Asn Gln Gln Leu
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Asn Tyr Phe Lys Asn Lys Leu Ser Lys Gln Asn Lys His Ala Lys Val
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Leu Glu Glu Ser Leu Glu Ile Met Ser Glu Lys Leu Arg Arg Thr Ala
465 470 475 480
Glu Asp Asn Arg Ile Val Arg Gln Arg Thr Lys Met Gln His Glu Gln
485 490 495
Asn Arg Glu Glu Met Asp Ala His Asp Arg Phe Phe Met Asp Ser Ile
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Lys Gln Ile His Glu Arg Arg Asp Ala Lys Glu Glu Asn Phe Glu Met
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Ile Asn Pro Ser Ser Asn Asp Asp Cys Arg Lys Arg Ala Glu Glu Val
545 550 555 560
Ser Ser Phe Ile Glu Phe Gln Glu Lys Glu Met Glu Glu Phe Val Glu
565 570 575
Glu Arg Glu Met Leu Ile Lys Asp Gln Glu Lys Lys Met Glu Asp Met
580 585 590
Lys Lys Arg His His Glu Glu Ile Phe Asp Leu Glu Lys Glu Phe Asp
595 600 605
Glu Ala Leu Glu Gln Leu Met Tyr Lys His Gly Leu His Asn Glu Asp
610 615 620
Asp
625